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Preparation of cells for immunofluorocytometric analysis of intracellular markers (FACS)

Stock solutions to have on hand:

- 10x PBS, pH 7.4
- 20% NaN_3
- 500mM EDTA, pH 8.0
- Formaldehyde (37.5%)
- 500 mM glycine
- 1% saponin

Solutions you will use:

- PBS
- PBS + 2mM EDTA
- PBS + 3.75% formaldehyde
- PBS + 100 mM glycine
- PBA (PBS + 1% BSA + 0.1% NaN_3)
- PBAsap (PBS + 0.1% BSA + 0.1% NaN_3 + 0.05% saponin)
- PBA + 1% formaldehyde

Note on this method: This is very similar to the preparation of cells for analysis of surface markers. The major differences are *fixation before labeling*, *permeabilization*, *all steps are performed at RT*, and *PBAsap is used for incubations*. Refer to the surface marker protocol for additional details.

Harvest cells:

Wash cell pellet twice with PBS. This and all other steps are performed at RT.

Fix and permeabilize:

Resuspend pellet in 50 - 100 µl PBS + 3.75% formaldehyde to fix cells. Incubate for 20 min. Wash once with PBS. Resuspend pellet in 500 µl PBS + 100 mM glycine to quench. Incubate for 10 min. Spin down cells and aspirate supernatant. Resuspend in 50 - 100 µl PBAsap to premeabilize. Incubate for 30 min.

Incubate with primary antibody:

Spin down the cells and resuspend in 50 - 100 µl ice cold PBAsap + primary antibody.

Incubate with secondary antibody, if necessary:

Spin down cells. Wash pellet 2 or 3 times by resuspension in 1 ml PBS. After washing, spin the cells down and resuspendin 50 - 100 µl PBAsap + secondary antibody. 30 min - 1 h.

Post-fixation:

Spin down cells. Wash pellet 2 or 3 times with 1 ml PBS. After washing, spin the cells down and resuspend in 100 µl PBA + 1% formaldehyde. The cells can be stored for up to one week at 4°C in the dark.

Preparation of samples for FACS:

The cells need to be diluted in PBS and filtered before they can be put into the FACS. The simplest way is to use tubes with cell strainer caps (Fisher Scientific catalog #08-771-23). Pop off the caps and add 500 µl PBS (RT) to each tube. Recap. One at a time, invert each tube to wet the inner surface of the filter and pipet the fixed cell suspension into the well of the cap. It works best if the filter is half-wet and you pipet the cells directly over the hanging drop of PBS. Don't puncture the filter. Your cells should fall right into the tube. For those that don't (it's a crap shoot), a very gentle spin will do the trick. Pop the caps back off and your samples are ready for FACSing.